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GATA1 mutation negative acute megakaryoblastic leukemia with acquired trisomy 21 presenting with extensive bone marrow necrosis in an adult: A case report and review of the literature☆,☆☆

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Abstract

Context: Acute megakaryoblastic leukemia (AMKL) is a rare myeloid leukemia, occurring in 1%–5% of all adult acute myeloid leukemia (AML) cases. AMKL is characterized by a leukemic blast population positive for factor VIII, CD41a, CD42b, and/or CD61 with extensive myelofibrosis. Although various cytogenetic abnormalities are commonly reported, the association between constitutional trisomy 21 and AMKL has been of particular interest, because of the near universal presence of *GATA1* mutation in such cases.

Objective and Design: We report a case of AMKL in an adult presenting with extensive bone marrow necrosis in which cytogenetic studies revealed three copies of chromosome 21 as part of a complex karyotype; however, sequencing of the *GATA1* gene revealed no mutation.

Results: The patient was an adult male who presented with extensive bone marrow necrosis, making definitive diagnosis difficult. Autopsy studies using a multimodality approach identified AMKL with a complex karyotype, including trisomy 21. Sanger sequencing of the *GATA1* gene showed a germline configuration without a mutation.

Conclusions: To our knowledge, this is the first reported case of an adult with AMKL with acquired trisomy 21 in which the GATA1 mutation was investigated and the second reported case of AMKL presenting with extensive bone marrow necrosis. We will present a diagnostic approach to AMKL in which extensive bone marrow necrosis renders examination of the bone marrow difficult. Furthermore, we will examine the absence of the GATA1 mutation in a case of AMKL with trisomy 21 in an adult.

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1. Introduction

Acute megakaryoblastic leukemia (AMKL) is rare in adults, occurring in about 1%–5% of all acute myeloid leukemia (AML) cases [1,2]. The diagnosis is typically made from morphologic and immunohistochemical (IHC) analysis of bone marrow aspirate and biopsy, which shows a leukemic blast population identified by IHC for factor VIII, CD41a, CD42b, and/or CD61 with at least $\geq 50\%$ of the blasts being megakaryoblasts, and often accompanied by extensive myelofibrosis demonstrated with a reticulin stain [1–3].

Unlike adults, AMKL is a relatively common form of AML in children, especially those with Down syndrome (DS) who have a 500-fold higher risk of developing AMKL [4]. Several studies have demonstrated that nearly all children with DS-associated AMKL have a mutation in the gene *GATA1*, a transcription factor essential for maturation and apoptosis of megakaryocytes [5–7]. Even though it is apparent that trisomy 21 is a key cytogenetic abnormality that predisposes to the development of AMKL in children, relatively few cases of adult AMKL without DS show a somatic gain of chromosome 21, such that there is no significant association between a somatic gain of chromosome 21 and the development of adult AMKL [8]. A search of the Mitelman database of chromosome aberrations and gene fusions in cancer showed 179 cases of adult AMKL and only 15 cases with a somatic gain of chromosome 21, and 10 of these 15 cases showed a complex karyotype (with at least 3 chromosomal aberrations). None of these cases showed somatic gain of chromosome 21 as a sole abnormality [9]. Only one reported case of adult AMKL has demonstrated a mutation in *GATA1*, and this patient did not have acquired trisomy 21 [6,10].

We report a case of a 54-year-old male that presented with pancytopenia and severe back pain. Cytogenetic analysis revealed a complex karyotype, including trisomy 21. The extensive marrow necrosis made for a challenging work-up; however, a diagnosis of AMKL was made at autopsy by examining extramedullary sites involved by leukemia with electron microscopy and IHC stains. This is only the second report of AMKL presenting with extensive bone marrow necrosis (the previous report was presented in 1984) [11], and the first report of an adult patient with AMKL and acquired trisomy 21 in which the GATA1 mutation was investigated.

2. Materials and methods

Approval was obtained from the institutional IRB at the University of Missouri Hospitals and Clinics, Columbia, Missouri. Bone marrow and paraffin embedded H&E slides were prepared by standard methods. Immunostaining was performed following the standard protocol on a Dako Immunostainer (Dako, Carpinteria, CA). Flow cytometric immunophenotyping was performed on a FacsCanto II flow cytometer (Becton-Dickinson, Franklin Lakes, NJ) using standard protocols. Cytogenetic studies were performed at Mayo Laboratories (Rochester, NY). Extracted DNA from autopsy tissue that was 85%–90% viable and 80% involved with AMKL was sent for *GATA1* Sanger sequencing (bidirectional sequencing of all coding exons (exons 2–6) of the *GATA1* gene) and was performed at Prevention Genetics (Marshfield, WI). The clinical sensitivity of this test is unknown and was validated by comparing 11.3 megabases of Sanger DNA sequence to NextGen sequencing generated at other labs [12]. Transmission electron microscopy was performed using standard methods. The electronic medical records were reviewed for pertinent demographic, past medical history, and physical exam findings.

3. Results

The patient is a 54-year-old male that presented to the emergency department with a one-week history of shortness of breath, fever, chills, night sweats, fatigue, anorexia, right upper quadrant abdominal pain, and lower back pain. Inpatient workup revealed anemia, thrombocytopenia, hepatosplenomegaly, and generalized lymphadenopathy. The differential diagnosis considered by hospital day 12 was hemophagocytic lymphohistiocytosis (ferritin level elevated to 38,459 ng/mL) versus hematopoietic/lymphoid neoplasm. Treatment with rituxan, etoposide, and dexamethasone was started on hospital day 13, due to concerns for hemophagocytic lymphohistiocytosis. During his hospital stay, he developed critical pancytopenia, respiratory failure, and renal failure. He expired on hospital day 17.

Wright's stained peripheral blood smears performed on hospital days 1 and 11 showed thrombocytopenia, anemia, and marked leukocytopenia with occasional blasts. The blasts had large round nuclei and prominent nucleoli with strongly basophilic cytoplasm and azurophilic granules. Flow cytometry performed on the peripheral blood revealed that approximately 1.5% of the cells represented an aberrant CD34+ myeloblast population. The clinical differential diagnosis included myelodysplasia and acute myeloid leukemia as well as other disorders associated with pancytopenia, including sepsis.

Bone marrow biopsies and aspirates were performed on hospital days 3 and 11. The aspirates showed scant cellularity and extensive necrosis. The core biopsies showed scattered immature pleomorphic atypical cells in a background of extensive necrosis (Fig. 1). The initial bone marrow biopsy was diagnosed as extensive necrosis with focal involvement by high-grade hematopoietic/lymphoid neoplasm. Flow cytometry performed on the peripheral blood on hospital day 11 revealed 8%–9% aberrant myeloblasts showing expression of CD7. The complete phenotype showed myeloblasts positive for CD45 (dim), CD34, CD117, CD33, CD13 (dim), and CD7 and

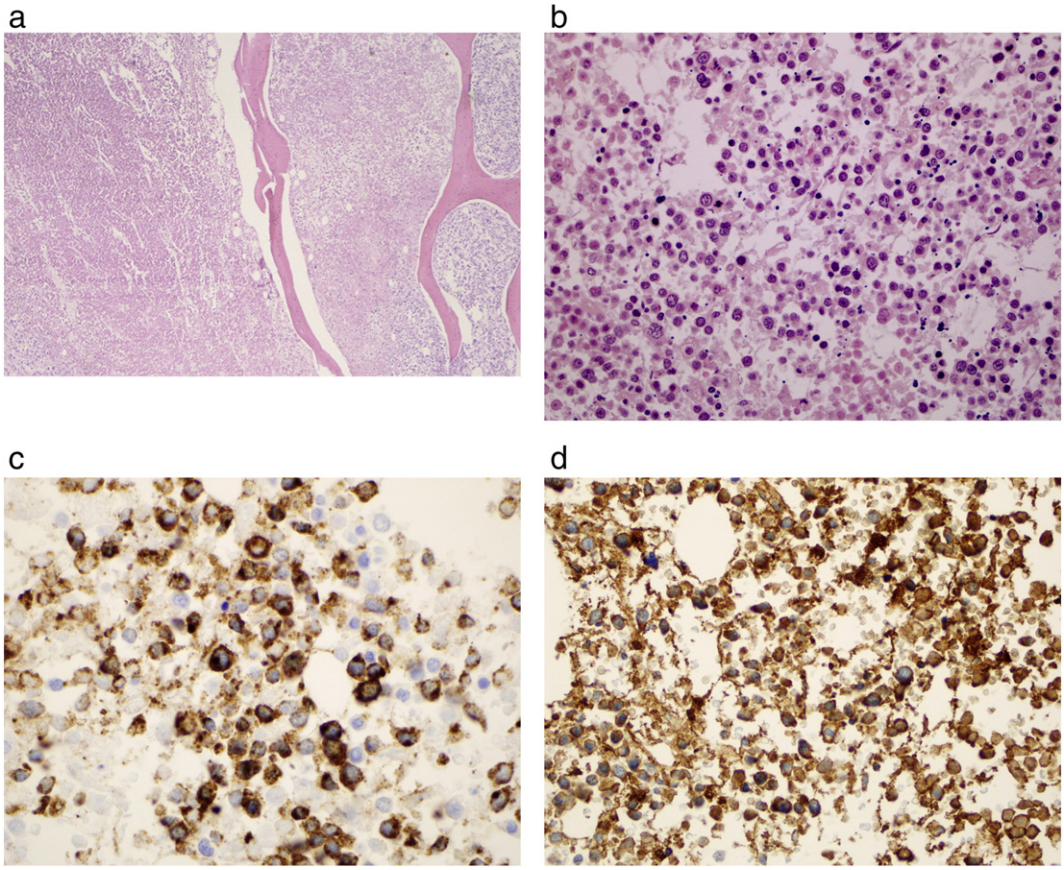

Fig. 1 (a) and (b), Bone marrow (H&E, 4× and 40×): extensive bone marrow necrosis was present. Scattered atypical cells are admixed, showing positivity for (c) CD34 (40×) and (d)Factor VIII (40×).

negative for HLA-DR, CD15, CD14, CD64, CD19, CD20, CD10, CD3, CD16, CD56, and CD11b. Cytogenetic analysis revealed an additional copy of chromosome 21 in nine of nine cells analyzed, consistent with a high-grade myeloid malignancy.

Complete karyotype report:
47–49,XY,add(2)(p13),-19,+21,add(21)(p11.2),add(21)(p13),+2mar[cp2]/
47–50,idem,der(11)t(11;11)(p15;q12),?add(20)(13.1),+1–3mar[cp7]

Autopsy studies revealed malignant cells infiltrating the bone marrow, spleen, lungs, kidneys, adrenal glands, lymph nodes, and liver (Figs. 2 and 3). Immunohistochemical studies (Fig. 4) were performed showing IHC positive for CD34, CD117, Factor VIII, CD42b, CD61, CD43, CD68, EMA, LCA, CD7 and negative for A103, S100, HMB45, ALK-1, CD1a, CD3, CD4, CD5, CD8, CD10, CD15, CD56, CD57, Herpes 8, EBV in the neoplastic cells in the liver and spleen confirming acute megakaryocytic leukemia. Electron microscopy showed extensive necrosis, but malignant cells that contained cytoplasmic granules with a double membrane, suggestive of alpha granules, were present (Fig. 5).

DNA was extracted from the malignant cell population from a frozen sample of spleen (85%–90% viability and 80% involved by AMKL), acquired at the time of autopsy. The DNA was analyzed with sequencing of the *GATA1* gene, which showed no variants from the reference sequence.

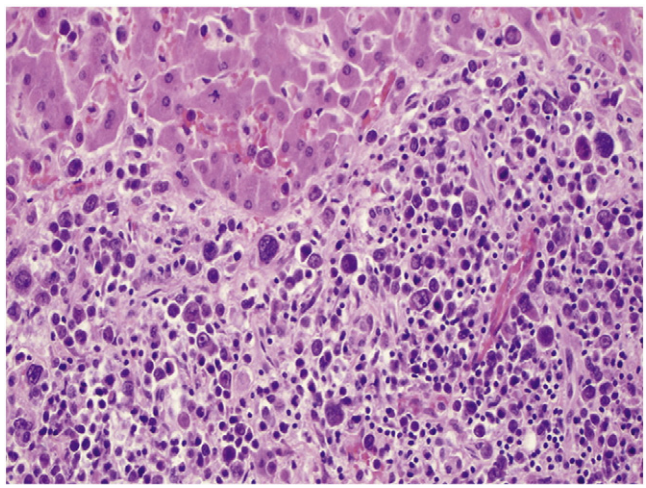

Fig. 2 Liver (H&E, 20×): disseminated leukemia infiltrating the liver. The malignant cells are markedly atypical and pleomorphic.

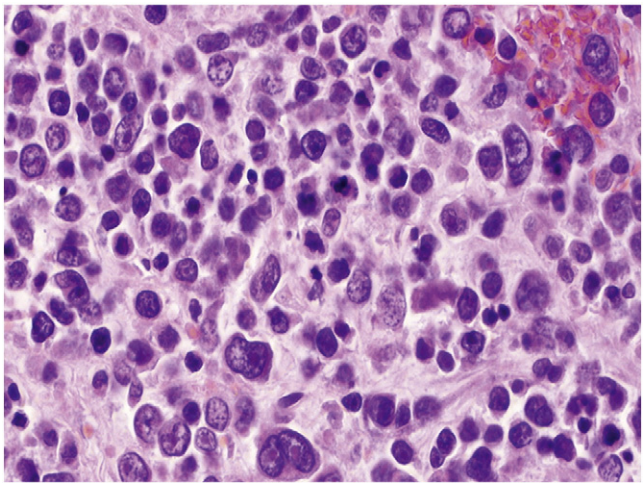

Fig. 3 Spleen (H&E, 40×): disseminated leukemia infiltrating the spleen. The malignant cells are markedly atypical and pleomorphic.

4. Discussion

AMKL is a rare leukemia comprising only 1%–5% of cases of AML [1,2]. Disease prevalence shows a bimodal age distribution: the median age for children is 12 months and the median age for adults is 58 years [13]. Patients present with cytopenias, especially thrombocytopenia, and may present with hepatosplenomegaly [14].

Bone marrow aspiration is often unsuccessful due to an extensively fibrotic marrow. In these cases, examination of the blasts in peripheral blood is most useful for diagnosis, since blasts are often present in the peripheral blood. Megakaryoblasts are typically medium to large (12–18 μm). The nucleus is round, slightly irregular or indented with fine chromatin and one to three nucleoli. The cytoplasm is basophilic, usually agranular, and may have blebs [1]. These features are not universal, however, and the morphologic features of megakaryoblasts are highly variable and are often not recognizable without special studies. A variety of cytologic features have been described for megakaryoblasts, and they can be categorized as follows as described by the Groupe Français d'Hématologie Cellulaire (GFHC) [13].

- Category 1A: The cells are large; the nucleus is round with fine chromatin; the nucleolus is prominent, but inconstant; the cytoplasm is basophilic and may have vacuoles.
- Category 1B: The cells are small-to-medium; the nucleus has dense chromatin; nucleoli are rare; cytoplasm is scant and may have azurophilic granulations.
- Category 2: This group has two types of blasts, resembling either the morphology of those in categories 1A or 1B. More than 50% of the blast cells are undifferentiated. Immunophenotypic studies are required for diagnosis.
- Category 3: The blasts are completely undifferentiated; megakaryoblastic assignment is impossible without immunophenotypic studies.

The blasts should express at least two megakaryocyte-associated antigens (CD41, CD42b, CD61, Von Willebrand factor, and/or factor VIII) and be negative for myeloperoxidase by flow cytometry and/or immunohistochemistry [1,3,14]. Dysplastic large platelets and hypogranular neutrophils may also be present [1,13].

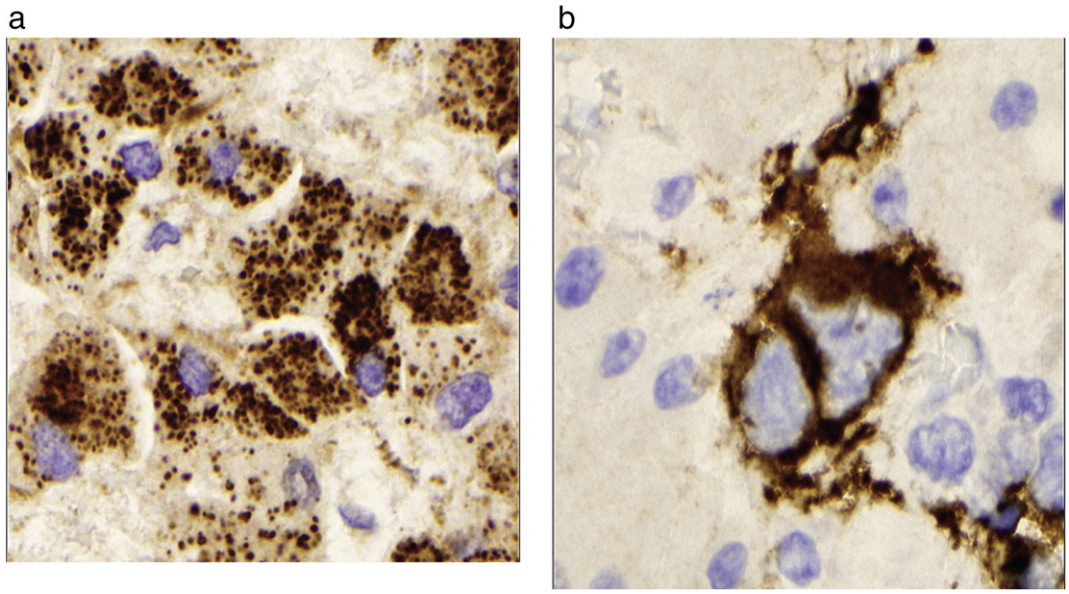

Fig. 4 (a) and (b), IHC (60×) for CD42b and CD61: malignant cells demonstrate positive staining for CD42b and CD61, suggesting megakaryocytic differentiation.

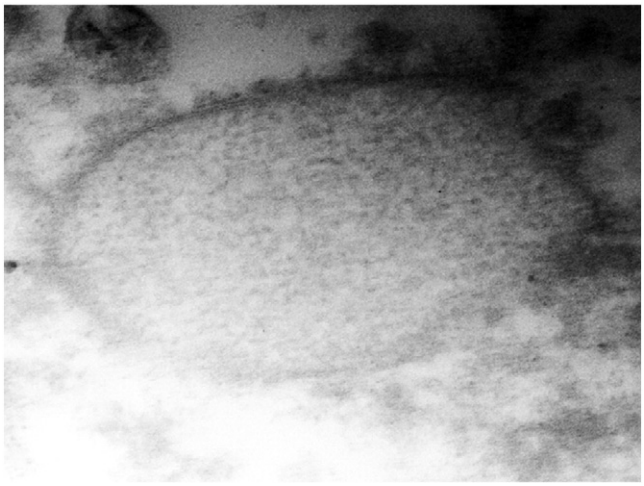

Fig. 5 Electron microscopy (200,000×) shows a membrane bound granule suggesting an alpha granule.

The prognosis for adults with AMKL is poor. Treatment regimens include various combinations of cytarabine (ara-C), an anthracycline, fludarabine, and topotecan. The complete remission rate is 43% to 50% with refractory disease reported in 62%, and a median overall survival time of 4.5–10.4 months. Even in patients that achieved complete remission, the median disease-free survival is only 6.5–10.6 months [2,13–16].

Extensive bone marrow necrosis is an uncommon entity, occurring in as little as 0.3% of bone marrow biopsies [17,18]. It is associated with a variety of etiologies: malignancy (91% of cases), infections, drugs, sickle cell disease, anorexia nervosa, diffuse intravascular coagulation, antiphospholipid syndrome, hemolytic uremic syndrome, and hyperparathyroidism [17]. Among these etiologies, hematologic malignancies represent the majority of cases (60% reported by Janssens et al. [17] and by Paydas et al. [19]) Several cases of bone marrow necrosis have been reported in association with AML, with bone marrow necrosis most commonly seen at the time of AML diagnosis [17,19,20]. For these reasons, it is essential to rule out malignancy when presented with a case of bone marrow necrosis [19].

Even though myeloid leukemias have been repeatedly described as an etiology of bone marrow necrosis, it remains a rare manifestation of AML (approximately 1.7% of AML cases) [20]. Furthermore, AMKL is a rare entity of AML, making this case particularly interesting, in which two rare entities intersected within the same patient. There has only been one case of AMKL with bone marrow necrosis reported in the literature [11]. AMKL is more typically known for its tendency to induce extensive bone marrow fibrosis rather than necrosis [2,15].

The pathogenesis of bone marrow necrosis is thought to result from a failure of microcirculation due to inflammatory mediators or mechanical obstruction. In cases of leukemia, the leukemic cells may outgrow the blood supply and mechanically obstruct the vascular structures. Furthermore, elaboration of prothrombotic cytokines, such as tumor necrosis factor (TNF), could be responsible for inducing a prothrombotic effect in the event of vascular damage [17,21].

Bone pain and fever are important presenting symptoms of bone marrow necrosis. Bone pain is the most common symptom, present in 78%–80% of cases, and may be disseminated or localized in the lower back [17,19]. Fever is seen in 68%–70% of cases, and may be associated with tissue necrosis or accompanying neutropenia [17,19]. Laboratory studies commonly show anemia, thrombocytopenia, leukoerythroblastosis, elevated lactic dehydrogenase, elevated alkaline phosphatase, elevated TNF and elevated fibrin degradation products [17,19,22].

On microscopic exam, the bone marrow aspirates show degenerated cells in a background of amorphous eosinophilic proteinaceous material. The degenerated cells have indistinct cell margins, shrunken and vacuolated cytoplasm, and a nucleus with pyknosis, karyorrhexis, or karyolysis. Trephine biopsies show degenerated cells in a background of gelatinous transformation and empty lacunae. The degenerated cells have similar cytologic features to those in the aspirate. Bone marrow necrosis is considered extensive when >50% of the biopsied marrow cavity shows necrosis [17].

Survival data show that adults with hematologic malignancies and bone marrow necrosis have a worse prognosis than those without bone marrow necrosis. The median survival for these patients ranges between a few weeks and 4 months [17–20]. Supportive care together with specific treatment for the underlying disease can induce spontaneous recovery of the bone marrow [17].

The presentation of bone marrow necrosis in this case represented a significant diagnostic challenge because of the absence of typical AMKL-associated morphologic features present in the bone marrow. Morphologic analysis of the extramedullary infiltrates, extensive IHC analysis, and even electron microscopy were required to identify features consistent with a diagnosis of AMKL.

Chromosomal abnormalities are found in 94% of patients with AMKL. Many of the karyotypes are complex (38.5% in children and 58.5% in adults) with a higher degree of complexity seen in adults. Recurring chromosomal abnormalities include trisomy 21 in 13.2%, t(1;22) in 20.8%, t(9;22) in 5.7%, t(3q21;3q26) in 11.3%, del 5q or del 7q in 18.9%, and inv(12) [13].

The association between trisomy 21 in Down syndrome (DS) and AMKL is well established in children, and children with DS have a 500-fold increased risk of developing AMKL compared with children without DS [4]. In just the past 12 years, studies have revealed that a mutation in *GATA1* on chromosome X is a common underlying mutation in DS-associated AMKL [5,6]. A 2002 study by Wechsler et al. [5] found that GATA1 was mutated in megakaryoblasts in each of 6 DS patients with AMKL. Rainis et al. [6] reported a case of monozygotic twins without DS who developed AMKL with acquired trisomy 21 in the blast cells. These twins had an identical mutation in *GATA1* as patients with

DS-associated AMKL. It was hypothesized that one twin acquired the mutation in utero and the preleukemic cells migrated to the other twin through embryonic blood connections. The mutation was not detected after the patients achieved remission. In contrast to DS-AMKL and pediatric non-DS AMKL, only one case has been reported of adulthood AMKL with mutated *GATA1* and this patient lacked a somatic gain of chromosome 21 [10].

How trisomy 21 compromises the integrity of the *GATA1* has yet to be determined. *RUNX1*, *ERG*, *ETS2*, and *GABP-alpha* are attractive candidate genes located on chromosome 21 [20–22]. *RUNX1* cooperates with *GATA1* during megakaryocytic differentiation [23–25]. Partial loss of *RUNX1* results in a significant increase of megakaryocyte progenitors and complete loss of *RUNX1* results in partial arrest of differentiation of megakaryocytes [23,24]. Abnormalities of *RUNX1* have been described in sporadic leukemia and in myeloid malignancies with acquired trisomy 21 [23,25].

ERG, *ETS2*, and *GABP-alpha* are members of the *ETS* transcription factor family and function in megakaryocytic differentiation. Overexpression of *ERG* and/or *ETS2* induces a switch in differentiation toward megakaryocyte lineage. Several studies have implicated their involvement in the pathogenesis of AMKL. *GABP-alpha* is expressed in the early stages of megakaryocytic maturation where it regulates the expression of genes involved in DNA synthesis and degradation of cell-cycle inhibitors [24,25].

Chromosome 21 also encodes several miRNAs that are overexpressed in patients with DS and implicated in megakaryocytic differentiation and AMKL oncogenesis. miR-99a is up-regulated during normal megakaryocytic differentiation, miR-155 and let-7c are expressed early in differentiation and are down-regulated during maturation, and miR-125b-2 is overexpressed in DS-associated AMKL [24,25].

In conclusion, this is the second reported case of AMKL presenting with extensive bone marrow necrosis. Bone marrow necrosis is often a harbinger for an underlying malignancy, often hematopoietic, and confers a poor prognosis, especially in adults. Therefore, this finding should instigate an expeditious search for malignancy so that a prompt treatment regimen may be initiated. In addition, ancillary testing such as flow cytometry, immunohistochemistry and cytogenetics will often be needed but may be difficult to interpret given the paucity of viable tumor.

The discovery of the *GATA1* mutation and its nearly universal presence in DS-associated AMKL has been an important discovery in explaining the leukemogenesis of the disease. While other studies have investigated for derangements of *GATA1* in adult cases of AMKL, it had not been investigated in a patient that also had a somatic gain of chromosome 21. While our patient showed an additional copy of chromosome 21, genetic sequencing did not reveal a *GATA1* mutation. This case casts doubt on the association of *GATA1* mutation in cases of adult AMKL with acquired trisomy 21. This finding necessitates further investigation of a candidate gene on chromosome 21, with *RUNX1* as a likely candidate, which could provide potential targets for therapy and surrogate markers for treatment response.

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References

[1] Arber DA, Brunning RD, et al. WHO Classification of Tumours of Haematopoietic & Lymphoid Tissues. Ch. 6: Acute myeloid leukaemia and related precursor neoplasms; 2008 136.
[2] Pagano L, Pulsoni A, et al. Acute megakaryoblastic leukemia: experience of GIMEMA trials. Leukemia 2002;16:1622-6.
[3] Foucar K, Reichard K, Czuchlewski D. Bone marrow pathology, 3rd ed. Ch. 18: Acute myeloid leukemia. Am Soc Clin Pathol 2010:376-431.
[4] Seawald L, Taub JW, Maloney KW, McCabe ER. Acute leukemias in children with Down syndrome. Mol Genet Metab 2012;107:25-30.
[5] Wechsler J, Greene M, McDevitt MA, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet 2002;32:148-52.
[6] Rainis L, Bercovich D, Strehl S, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood 2003;102:981-6.
[7] Zheng R, Blobel GA. GATA transcription factors and cancer. Genes Cancer 2011;1:1178-88.
[8] Hama A, Muramatsu H, et al. Molecular lesions in childhood and adult acute megakaryoblastic leukaemia. Br J Haematol 2012;156:316-25.
[9] Mitelman F, Johansson B, Mertens F, editors. Mitelman database of chromosome aberrations and gene fusions in cancer; 2015 http://cgap.nci.nih.gov/Chromosomes/Mitelman.
[10] Harigae H, Xu G, et al. The GATA1 mutation in an adult patient with acute megakaryoblastic leukemia not accompanying Down syndrome. Blood 2004;108:3242-3.
[11] Wang SE, Fligiel S, Naeim F. Acute megakaryocytic leukemia following chemotherapy for a malignant teratoma. Arch Pathol Lab Med 1984;108:202-5.
[12] Chicka M. Prevention Genetics: Thrombocytopenia via the GATA1 Gene, Methods and Pricing. https://www.preventiongenetics.com/clinical-dna-testing/test/thrombocytopenia-via-the-gata1-gene/102/; 2012.
[13] Duchayne E, Fenneteau O, et al. Acute megakaryoblastic leukaemia: a national clinical and biologic study of 53 adult and childhood cases by the Groupe Français d'Hématologie Cellulaire (GFHC). Leuk Lymphoma 2002;44:49-58.
[14] Orazi A. Histopathology in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes, and myelodysplastic/myeloproliferative diseases. Pathobiology 2007;74:97-114.
[15] Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood 2000;96:2405-11.
[16] Oki Y, Kantarjian HM, et al. Adult acute megakaryocytic leukemia: an analysis of 37 patients treated at M.D. Anderson Cancer Center. Blood 2006;107:880-4.
[17] Janssens AM, Offner FC, Van Hove WZ. Bone marrow necrosis. Cancer 2000;88:1769-80.
[18] Elgamal BM, Rashed RA, Raslan HN. Prevalence of bone marrow necrosis in Egyptian cancer patients referring to the National Cancer Institute. J Egypt Natl Cancer Inst 2001;23:95-9.

[19] Paydas S, Ergin M, Baslamisli F, et al. Bone marrow necrosis: clinicopathologic analysis of 20 cases and review of the literature. Am J Hematol 2002;70:300-5.
[20] Forrest DL, Mack BJ, Nevill TJ, et al. Bone marrow necrosis in adult acute leukemia and non-Hodgkin's lymphoma. Leuk Lymphoma 2000;38:627-32.
[21] Kato M, Kikuchi A, Oshima K, et al. Pediatric acute lymphoblastic leukemia initially presenting with bone marrow necrosis. Jpn J Clin Hematol 2007;48:140-3.
[22] Seki Y, Koike T, Aoiki S, et al. Bone marrow necrosis with dyspnea in a patient with malignant lymphoma and plasma levels of thrombomodulin, tumor necrosis factor-alpha, and D-dimer. Am J Hematol 2002;70:250-3.
[23] Berger R, Busson M, et al. Acute megakaryoblastic leukemia and loss of the RUNX1 gene. Cancer Genet Cytogenet 2006;164:71-3.
[24] Roy A, Roberts I, Vyas P. Acute megakaryoblastic leukaemia (AMKL) and transient myeloproliferative disorder (TMD) in Down syndrome: a multi-step model of leukamogenesis. Br J Haematol 2009;147:3-12.
[25] Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. Blood 2009;113:2619-28.